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Analysis of aqueous humour proteins of eyes with and without pseudoexfoliation syndrome

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Abstract Pseudoexfoliation syndrome (PEX) has been suggested to represent a blood-aqueous barrier impairment leading to a higher protein content in aqueous humour of eyes with PEX. However, the nature of a prospective PEX protein has not yet been described. We set out to re-evaluate protein content and examine protein composition for prospective PEX protein candidates in aqueous humour of eyes with PEX syndrome. Aqueous humour of 52 patients with PEX and 38 without PEX signs was sampled during cataract or glaucoma surgery. Total aqueous protein concentration in the samples was analysed in 43 PEX specimens and 32 non-PEX specimens according to Bradford. Aqueous protein composition of all samples was determined by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS PAGE) and silver staining. Screening for amyloids was performed in nine PEX samples and six non-PEX samples by Congo Red staining and polarised light microscopy. Aqueous protein concentration was not significantly increased in PEX eyes in comparison with non-PEX eyes. Furthermore, we could not detect any characteristic difference in protein band sizes of the two groups after SDS PAGE. However, we were able to show the presence of amyloid exclusively in aqueous humour of PEX patients. *Conclusion:* our results do not confirm a generally higher protein concentration in pseudoexfoliation syndrome eyes. This does not necessarily contradict a blood-aqueous barrier impairment but illustrates the variance in protein concentration between and within the two groups. No characteristic protein band allocatable to pseudoexfoliation syndrome proteins could be detected in any of the samples. However, our findings support the theory that the pseudoexfoliation syndrome is associated with an amyloid of a serum protein.

Introduction

Pseudoexfoliation syndrome (PEX) is a disorder characterised by a widespread production and deposition of a distinctive fibrillar material in the eye and in various organs such as skin, heart, lungs, liver, kidney and meninges [26]. In the eye, PEX is characterised by the presence of this material on the pupillary border, the anterior lens capsule, both surfaces of the iris, ciliary body, zonules, in the anterior chamber angle, and occasionally on the posterior surface of the cornea [12]. A simultaneous mechanism of local production of the material by anterior segment tissues and transport via the aqueous humour has been suggested [20]. There is strong evidence that PEX represents an ocular manifestation of a systemic disorder and that PEX material may be synthesised outside the eye before it becomes evident in the anterior segment [27].

One of the most important clinical features of PEX is the frequent association with open-angle glaucoma. In

addition, the incidence of cataract is high in eyes with PEX [30,31]. Patients with PEX are at an approximately ten fold increased risk of developing a rather aggressive type of secondary open-angle glaucoma and a secondary angle-closure glaucoma [23]. Furthermore, PEX is presently the most common cause of open-angle glaucomas world-wide [22].

Despite its clinical significance, the exact composition of the abnormally produced material remains unknown. Studies performed on the nature of PEX material revealed an association with amyloid [17,19], but other authors have shown the principal basement membrane components heparan and chondroitin sulphate proteoglycan, and entactin/nidogen [25], and particularly the elastic fibre components elastin, amyloid P, vitronectin, and fibrillin-1 to be integral constituents of PEX material [10, 28,29].

It has also been demonstrated that an impairment of the blood-aqueous barrier is frequently associated with PEX [5,8] leading to an altered composition of aqueous humour. Increased aqueous protein concentrations were reported as compared to age-matched controls [5, 6, 7, 13] as well as changes in the levels of alpha1-lipoprotein and caeruloplasmin [2], transferrin [4] and fibronectin [32]. Electrophoretic analyses of aqueous proteins demonstrated either a prominent band at 12.5 kDa [5] or at 16.3 kDa [20] in aqueous humour of PEX patients.

It was the aim of this study to re-evaluate the protein content and to examine the protein composition for the possible presence of PEX-specific proteins in aqueous humour of eyes with PEX syndrome. In addition, we screened aqueous humour of patients with and without PEX syndrome for the presence of amyloid proteins.

Materials and methods

Materials

Aqueous humour was obtained during intraocular surgery from 52 eyes of 52 patients who attended the University Eye Clinics in Rostock and Erlangen (eight with secondary open-angle glaucoma, one with primary open-angle glaucoma, 43 with cataract, age range 67–90 years, mean 77.1 years, male/female ratio 1:1.18) with the characteristic clinical signs of PEX and from 38 eyes of 38 patients (all with cataract, two with an additional primary open-angle glaucoma, age range 52–82 years, mean 70.4 years, male/female ratio 1:1.53) without any clinical signs of PEX.

Methods

Total protein concentrations were determined for 43 of the 52 aqueous humour samples of PEX patients and 32 of the 38 samples of control patients using a Bradford assay (BioRad, Germany). Aqueous protein composition of 10 µl of the same samples was determined by 15% sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS PAGE) and silver staining according to standard protocols. The remaining nine PEX specimens and six non-PEX specimens were used for amyloid screening. A volume of 10 µl of each sample was streaked on glass slides and air-dried. Screening for amyloid was performed by 0.1% Congo Red staining and polarised light microscopy according to Romhányi [24].

Results

Total aqueous protein concentrations were not significantly higher in PEX eyes (0.23±0.19 mg/ml, range 0.008–1.001 mg/ml) than in the non-PEX group (0.26±0.23 mg/ml, range 0.038–0.875 mg/ml) (P>0.5, Student's t-test). Within the PEX group the aqueous humour of the 43 cataract patients showed a lower mean protein concentration (0.237 mg/ml) than that of the eight patients with secondary open-angle glaucoma

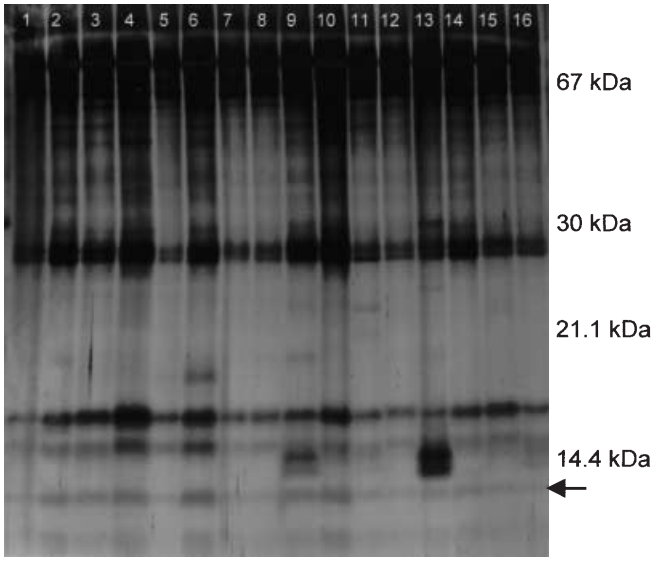

Fig. 1 SDS-PAGE of aqueous humour of eight patients with PEX (lanes 1–8) and eight patients without PEX (lanes 9–16). Note the 12.5 kDa band (*arrow*) visible in all specimens. (*M* marker)

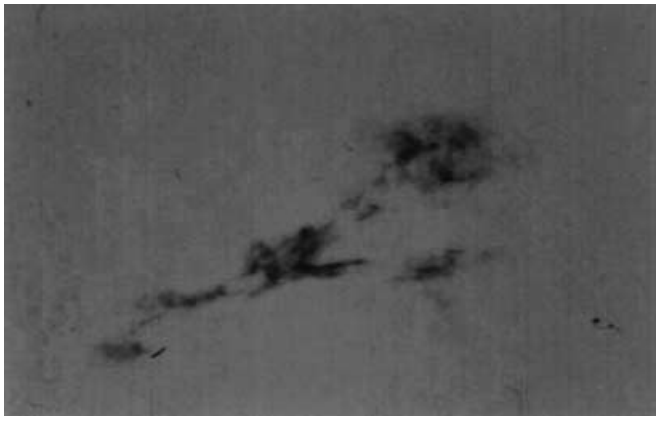

Fig. 2 Positive amyloid reaction in aqueous humour of an eye with PEX (Congo red, ×200)

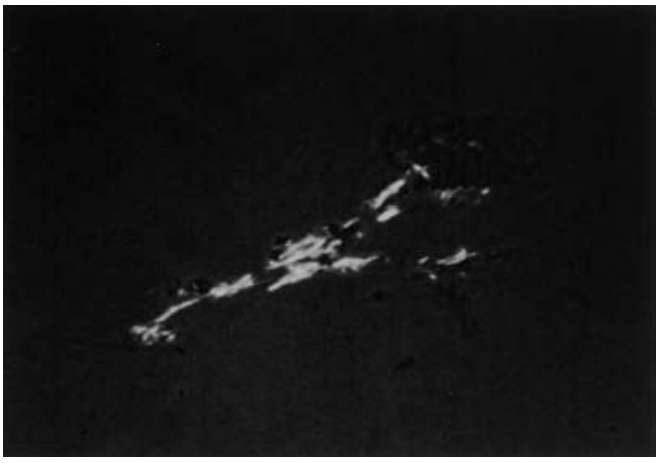

Fig. 3 Positive amyloid reaction in aqueous humour of an eye with PEX (Congo red, polarised light, ×200)

(0.380 mg/ml). However, this difference was not significant ($P>0.05$, Student's t-test). SDS-PAGE did not reveal any significant band differences between both groups (Fig.1).

Amyloid exhibiting typical green birefringence was demonstrated in the presence of Congo Red under cross-polarised light in all nine examined aqueous humour samples of PEX patients (Fig. 2, Fig. 3). The six non-PEX samples were negative for amyloid.

Discussion

PEX has been suggested to represent the intraocular manifestation of a systemic disorder, characterised by grey dandruff-like material deposited in the anterior part of the eye. Many clinical studies have demonstrated an impaired blood-aqueous barrier function in eyes with PEX [5, 7, 8, 9, 15] which may lead to protein leakage and increased total aqueous protein concentration. Iris fluorescein angiographic and electron microscopic studies [1, 25] could show that the origin of the increased aqueous proteins is mainly the iris vasculature [1, 6, 8, 16].

The results of our study do not confirm a significant mean increase in total aqueous protein concentration in eyes with PEX. This finding is in accordance with Ringvold and co-workers [21] who described increased protein concentrations only in lens capsule specimens, but not in aqueous humour samples of PEX eyes. However, our findings contradict the results of Küchle and co-workers [6] who found significantly higher protein concentrations in aqueous humour of patients with PEX with and without glaucoma. However, the protein concentrations measured in this study showed considerable interindividual variations both in PEX eyes and in control eyes. This finding does not necessarily contradict a blood-aqueous barrier impairment but rather illustrates the variance in protein concentrations between and within the two groups. We conclude that PEX is not necessarily characterised by increased protein concentrations in aqueous humour. Aqueous protein concentrations appear to be subject to high interindividual variations depending on patient and development of the disease rather than being clearly related to the PEX process. Since the aqueous protein concentrations may depend on the stage of disease progression, a possible explanation for these differences might be the fact that the majority of aqueous samples analysed in this study was obtained from patients with PEX syndrome without glaucoma.

The exact composition of PEX material and the pathogenesis of PEX are still unclear. So far, no PEX-specific proteins could be identified with certainty in the aqueous humour, although two electrophoretic studies have suggested proteins of a molecular weight of 12.5 kDa and 14.4/16.3 kDa as possible candidates [6, 21]. The results of this study confirmed a protein band of 12.5 kDa, which was, however, present in all samples independent of PEX. However, this band had been only observed in 56% of PEX eyes in the study by Küchle et al. [6]. A band of 14.4 kDa only occurred in two non-PEX aqueous humour samples, whereas a 16.3 kDA band was found in all samples. Thus, our results do not confirm the presence of one or several specific proteins in the aqueous humour of PEX eyes. However, the possibility of the presence of proteins which are not solubilised by standard methods or not detectable with one-dimensional SDS-PAGE electrophoresis cannot be ruled out.

Although some cases with PEX and primary amyloidosis have been described [11], the relationship of PEX material with amyloid has always been a matter of much debate. Whereas a few studies suggested PEX material to be a type of amyloid [17, 19], most studies examining the nature of PEX material by Congo Red staining or immunohistochemistry with specific antibodies have yielded negative reactions for amyloid [3, 14, 18]. Thus, the amyloid theory lacked any conclusive evidence. In the present study, clear evidence for the presence of amyloid proteins in the aqueous humour of PEX eyes but not of control eyes was provided by Congo Red staining. Therefore, these findings indicate an association of PEX with amyloid formation and further substantiate the amyloid theory on the pathogenesis of PEX syndrome. However, the exact type of amyloid in the aqueous humour and the deposition of amyloid in anterior segment tissues of PEX eyes remain to be elucidated in future studies.

References

1. Asano N, Schlötzer-Schrehardt U, Naumann GO (1995) A histopathologic study of iris changes in pseudoexfoliation syndrome. Ophthalmology 102:1279–1290
2. Baba H (1982) Investigation of the pathogenesis of glaucoma capsulare with special discussion of alpha 1 Lp and Cp in aqueous humor. Graefes Arch Clin Exp Ophthalmol 218:283–286
3. Dark AJ, Streeten BW, Cornwall CC (1977) Pseudoexfoliative disease of the lens: a study in electron microscopy and histochemistry. Br J Ophthalmol 61:462–472
4. Koliakos GG, Konstas AG, Dimitrakoulias N, Triantos A, Kardasopoulos A, Dimitriadou A, Trakatellis AC (1996) Possible role of transferrin in exfoliation syndrome. Acta Ophthalmol Scand 74:155–159
5. Küchle M, Nguyen NX, Horn F, Naumann GO (1992) Quantitative assessment of aqueous flare and aqueous 'cells' in pseudoexfoliation syndrome. Acta Ophthalmol (Copenh) 70:201–208
6. Küchle M, Ho TS, Nguyen NX, Hannappel E, Naumann GO (1994) Protein quantification and electrophoresis in aqueous humor of pseudoexfoliation eyes. Invest Ophthalmol Vis Sci 35:748–752
7. Küchle M, Nguyen NX, Hannappel E, Naumann GO (1995) The blood-aqueous barrier in eyes with pseudoexfoliation syndrome. Ophthalmic Res 27[Suppl 1]:136–142
8. Küchle M, Vinores SA, Mahlow J, Green WR (1996) Blood-aqueous barrier in pseudoexfoliation syndrome: evaluation by immunohistochemical staining of endogenous albumin. Graefes Arch Clin Exp Ophthalmol 234:12–18
9. Laurell CG, Zetterstrom C, Philipson B, Syren-Nordqvist S (1998) Randomized study of the blood-aqueous barrier reaction after phacoemulsification and extracapsular cataract extraction. Acta Ophthalmol Scand 76:573–578
10. Li ZY, Streeten BW, Wallace RN (1988) Association of elastin with pseudoexfoliative material: an immunoelectron microscopic study. Curr Eye Res 7:1163–1172
11. Meretoja J, Tarkkanen A (1977) Occurence of amyloid in eyes with pseudoexfoliation. Ophthalmic Res 9:80–91
12. Meyer E, Haim T, Zonis S, Gidoni O, Gitay H, Levanon D, Nir I (1984) Pseudoexfoliation: epidemiology, clinical and scanning electron microscopic study. Ophthalmologica 188:141–147
13. Moreno-Montanes J, Lajara Blesa J (1995) IgG, albumin and total IgG index in the aqueous humour of eyes with pseudoexfoliation syndrome. Acta Ophthalmol Scand 73:249–251
14. Morrison JC, Green WR (1988) Light microscopy of the exfoliation syndrome. Acta Ophthalmol Suppl 184:5–27
15. Nguyen NX, Küchle M, Martus P, Naumann GO (1999) Quantification of blood–aqueous barrier breakdown after trabeculectomy: pseudoexfoliation versus primary open-angle glaucoma. J Glaucoma 8:18–23
16. Parodi MB, Bondel E, Saviano S, Ravalico G (2000) Iris indocyanine green angiography in pseudoexfoliation syndrome and capsular glaucoma. Acta Ophthalmol Scand 78:437–442
17. Repo LP, Naukkarinen A, Paljarvi L, Terasvirta ME (1996) Pseudoexfoliation syndrome with poorly dilating pupil: a light and electron microscopic study of the sphincter area. Graefes Arch Clin Exp Ophthalmol 234:171–176
18. Ringvold A (1993) Update in etiology and pathogenesis of the pseudoexfoliation syndrome. New Trends Ophthalmol 8:177–180
19. Ringvold A, Husby G (1973) Pseudoexfoliation material-an amyloid-like substance. Exp Eye Res 17:289–299
20. Ringvold A, Vegge T (1971) Electron microscopy of the trabecular meshwork in eyes with exfoliation syndrome. (Pseudoexfoliation of the lens capsule). Virchows Arch A Pathol Pathol Anat 353:110–127
21. Ringvold A, Husby G, Pettersen S (1989) Electrophoretic study of proteins associated with pseudo-exfoliation syndrome. Acta Ophthalmol (Copenh) 67:724–726
22. Ritch R (1994) Exfoliation syndrome and occludable angles. Trans Am Ophthalmol Soc 92:845–944
23. Ritch R, Schlötzer-Schrehardt U (2001) Exfoliation Syndrome. Surv Ophthalmol 45:265–315
24. Romhanyi G (1979) Selective demonstration of amyloid deposits and methodical possibilities of analysis of their ultrastructural differences. Zentralbl Allg Pathol 123:9–16
25. Schlötzer-Schrehardt U, Dörfler S, Naumann GO (1992) Immunohistochemical localization of basement membrane components in pseudoexfoliation material of the lens capsule. Curr Eye Res 11:343–355
26. Schlötzer-Schrehardt UM, Koca MR, Naumann GO, Volkholz H (1992) Pseudoexfoliation syndrome. Ocular manifestation of a systemic disorder? Arch Ophthalmol 110:1752–1756
27. Schlötzer-Schrehardt U, Küchle M, Dörfler S, Naumann GOH (1993) Pseudoexfoliative material in the eyelid skin of pseudoexfoliation-suspect patients: a clinico-histopathological correlation. Germ J Ophthalmol 2:51–60
28. Schlötzer-Schrehardt U, von der Mark K, Sakai LY, Naumann GOH (1997) Increased extracellular deposition of fibrillin-containing fibrils in pseudoexfoliation syndrome. Invest Ophthalmol Vis Sci 38:970–984
29. Streeten BW, Gibson SA, Dark AJ (1986) Pseudoexfoliative material contains an elastic microfibrillar-associated glycoprotein. Trans Am Ophthalmol Soc 84:304–320
30. Sugar S (1984) Pigmentary glaucoma and the glaucoma associated with the exfoliation-pseudoexfoliation syndrome: update. Robert N. Shaffer lecture. Ophthalmology 91 307–310
31. Tarkkanen A (1962) Pseudoexfoliation of the lens capsule. Acta Ophthalmol 71:1–98
32. Vesaluoma M, Mertaniemi P, Mannonen S, Lehto I, Uusitalo R, Sarna S, Tarkkanen A, Tervo T (1998) Cellular and plasma fibronectin in the aqueous humour of primary open-angle glaucoma, exfoliative glaucoma and cataract patients. Eye 12: 886–890